Interaction of Volatile Anesthetics with Human Kv Channels in Relation to Clinical Concentrations

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Background: Recent evidence shows that inhibition of human Kv3 channels by intravenous anesthetics occurs at clinical concentrations. The effects of volatile anesthetics on these human ion channels are unknown. This study was designed to establish whether minimum alveolar concentrations (MAC) of halothane, enflurane, isoflurane, and desflurane exhibit effects on Kv3 channels. To obtain an indication whether these findings may be specific to Kv3 channels, the effects of enflurane and isoflurane on human Kv1.1 channels were also investigated.

Methods: Kv3 channels natively expressed in SH-SY5Y cells and Kv1.1 channels expressed in HEK293 cells were measured with the whole cell patch clamp technique by standard protocols. Concentrations of volatile anesthetics were determined by gas chromatography.

Results: Halothane, enflurane, isoflurane, and desflurane reversibly inhibited Kv3 channels in a concentration-dependent manner. Concentrations at half-maximal effect (IC50 values) ranged between 1,800 and 4,600 μM. Hill coefficients were between 1.7 and 2.5. IC50 values for inhibition of Kv1.1 channels were 2,800 and 5,200 μM, and Hill coefficients were 3.9 and 5.6 for enflurane and isoflurane, respectively.

Conclusion: Volatile anesthetics inhibit human Kv3 channels at clinical concentrations. At 1–3 MAC, inhibition would account on average for 2–12%. Inhibition would be highest with enflurane (between 3% and 22%) and lowest with isoflurane (between 0.2% and 3%). Kv1.1 channels would only be inhibited by enflurane at clinical concentrations (2% at 2 MAC and 8% at 3 MAC). Whether the degree of K channel inhibition by volatile anesthetics may contribute to their clinical action needs further study.

VOLTAGE-DEPENDENT K channels constitute a heterogeneous group of membrane proteins that are important for various cellular functions.1,2 Alterations of these ion channels result in complex cellular changes.3,4 In a previous study, we described the effects of several intravenous anesthetics on human neuronal voltage-dependent Kv3 channels.5 In contrast to expectations, the intravenous anesthetics inhibited Kv3 channels already at clinically relevant concentrations, and concentrations at half-maximal effect (IC50 values) for inhibition of Kv3 channels by intravenous anesthetics correlated with their clinical concentrations.

The action of volatile anesthetics on human voltage-dependent K channels has not been investigated. It is unknown whether these ion channels may be a molecular target of inhalation anesthetics at clinical concentrations. In light of our previous findings, the current study was designed to extend our observations with intravenous anesthetics to the commonly used inhalation anesthetics.

The study examined the action of halothane, enflurane, isoflurane, and desflurane on human Kv3 channels. Concentration-dependent effects were established to relate concentrations of in vitro effects to minimum alveolar concentrations (MAC). To obtain an indication of whether these findings may be specific to Kv3 channels, they were repeated for enflurane and isoflurane on human Kv1.1 channels stably transfected in HEK293 cells.

Material and Methods

Cell Culture and Electrophysiologic Recordings

SH-SY5Y cells6 were grown as previously described.5 HEK293 cells stably expressing Kv1.1 channels were grown in nonconfluent monolayer using DMEM nutrient mix F12 (Life Technologies, Paisley, Scotland) at 37°C with 95% air and 5% CO2. Natively expressed voltage-dependent K channels7 in SH-SY5Y cells and Kv1.1 channels were recorded with the whole cell patch clamp technique8 as described previously. The holding potential in all experiments was −80 mV; the test potentials were rectangular pulses with a duration of 100 ms increasing from −50 mV to +70 mV in 10-mV steps for Kv3 channels and from −70 mV to +50 mV in 10-mV steps for Kv1.1 channels. The effect of the drugs was recorded during the superfusion of the cells, and wash-out of drug effect was measured during the superfusion of the cells with drug-free solution. The drugs were purchased from Abbot (Wiesbaden, Germany; halothane, enflurane, isoflurane) and Pharmacia (Erlangen, Germany; desflurane). The recorded signal was filtered at 3 kHz, digitized using an analog-to-digital converter (Digidata 1200; Axon Instruments, Foster City, CA), and stored on a 586 IBM-compatible personal computer with a sampling rate of 10 kHz for later analysis. The concentration of the volatile anesthetic agents in the test solution was measured during each individual experiment by gas chromatography (GC-14B; Shimatzu, Duisburg, Germany).
of experiments. IC50 values and Hill coefficients are concentration-dependent effects on Gmax of Kv3 channels according to the Hill function.

Concentration for half-maximal inhibition (IC50 values) and Hill coefficients resulted from the fit of the concentration-dependent effects on Gmax of Kv3 channels according to the Hill function.

Concentration gradient), and voltage was corrected using the measured series resistance and compensation level. The conductance-voltage data were fitted to a Boltzmann function of the form:

\[ G(V) = \frac{G_{\text{max}} - G_{\text{min}}}{1 + \exp(-z_a F (V - V_{\text{mid}})/RT)} + G_{\text{min}} \]  (1)

where Gmax and Gmin are the maximum and minimum conductances measured for the dataset; Vmid is the voltage corresponding to 50% of maximum conductance; za is the effective gating charge; and R, T, and F have their usual meanings. Inhibition was defined as 1 minus the ratio of Gmax in the presence of the drug to the mean of the current before and after application of the drug. The concentration-response curves were fitted with the equation

\[ c/e_{\text{max}} = c^\gamma [1/C_{50} + c^\gamma] \]  (2)

using nonlinear regression analysis (SAS Institute, Cary, NC). Here, c = effect, cmax = maximal effect, c = anesthetic concentration, and γ = Hill coefficient. Concentration-response data for inhibition of Gmax were fitted with cmax = 1. The number is always the number of experiments. IC50 values and Hill coefficients are given as mean ± standard error of the mean as specified by the Sigma-Plot software (Kandel GmbH, Erkrath, Germany). All other data are given as mean ± SD unless stated otherwise. The values of minimum alveolar concentrations and octanol-water coefficients (table 1) were taken from literature sources.

**Data and Statistical Analysis**

The peak outward potassium current in a trace was determined by fitting the current to a two-exponential time course (activation and inactivation) and determining the maximal amplitude of the fit. Peak currents were converted to conductances using the Nernst potential for potassium (−85 mV for the standard K+ concentration gradient), and voltage was corrected using the measured series resistance and compensation level. The conductance-voltage data were fit to a Boltzmann function.

\[ G(V) = \frac{G_{\text{max}} - G_{\text{min}}}{1 + \exp(-z_a F (V - V_{\text{mid}})/RT)} + G_{\text{min}} \]  (1)

**Results**

The Kv3 current traces in figure 1 show the effects of the drugs investigated in this study. All volatile anesthetics reversibly inhibited these potassium channels negatively expressed in human neuroblastoma SH-SY5Y cells. The halogenated ethers enflurane, isoflurane, and desflurane, but not the halogenated alkane halothane, accelerated macroscopic current decline (fig. 1A). The effects of the anesthetics on Kv3 channels were measured as inhibition of the maximal whole cell conductance (Gmax; for details see Methods). During control conditions, Gmax was 9.1 ± 3.9 nanosiemens (n = 89). Inhibition of Gmax was concentration dependent and reversible at all concentrations. Inhibition was described mathematically by Hill functions (fig. 1B, table 1). The IC50 values for inhibition of Gmax by the volatile anesthetics were between 1,800 μM for halothane and 4,450 μM for desflurane. The Hill coefficients were between 1.7 and 2.5.

With the intention to clarify whether the results reported may be unique to Kv3 channels, the actions of enflurane and isoflurane on the whole cell conductance of human Kv1.1 channels were also examined (fig. 2A). These ion channels were stably transfected in HEK293 cells. During control conditions, Gmax was 23 ± 13 nanosiemens (n = 77). Both anesthetics reversibly inhibited Kv1.1 channels and accelerated macroscopic current decline (fig. 2A). Inhibition was concentration dependent (fig. 2B) and reversible at all concentrations. IC50 values for inhibition of Gmax were 2,760 ± 369 (n = 22) for enflurane and 5,160 ± 219 (n = 29) for isoflurane. Hill coefficients were 3.9 ± 1.2 and 5.6 ± 0.8 for enflurane and isoflurane, respectively (fig. 2B).

**Discussion**

The volatile anesthetic agents halothane, enflurane, isoflurane, and desflurane reversibly inhibit human neuronal Kv3 channels in a concentration-dependent manner. The current study allows extending our observations with intravenous anesthetics to the commonly used inhalation anesthetics.

Although the volatile anesthetics and the intravenous anesthetics both inhibit Kv3 channels in a concentration-dependent and reversible manner, the concentration-response curves and the ratios of IC50 values to clinical concentrations differ between both groups of anesthetic.
agents. Whereas IC$_{50}$ values of intravenous anesthetics are on average 22-fold greater than of the MAC equivalent of intravenous anesthetics (CP$_{50}$), half-maximal inhibition of Kv3 channels by volatile anesthetics occurs at concentrations on average 9-fold more than 1 MAC. Whereas intravenous anesthetics inhibit Kv3 channels with Hill coefficients close to unity (mean value 1.1, n = 7), the Hill coefficient for inhibition by volatile anesthetics is 2.1 (mean value, n = 4). Despite these differences, inhibition of Kv3 channels by both groups of agents is on average similar at clinical concentrations. As calculated from the Hill functions at 1 MAC, human Kv3 channels would be inhibited by the volatile anesthetics by 2% (2.9% by intravenous anesthetics at CP$_{50}$ values). At 1 MAC or more, inhibition of Kv3 channels would be highest with enflurane (between 3% and 22% between 1 and 3 MAC) and lowest with isoflurane (between 0.2% and 3% between 1 and 3 MAC).

Fig. 1. (A) Superimposed traces of voltage-dependent Kv3 channels activated by depolarizing steps from a holding potential of −80 mV to test potentials ranging from −50 mV to +70 mV. The interpulse duration was 1 s. Shown are the current traces during control conditions, during the influence of the anesthetic agents on the K currents, and after the drugs were washed out. The concentrations used were 1,800 µM enflurane, 1,980 µM isoflurane, 1,100 µM halothane, and 6,100 µM desflurane. (B) Concentration–response curves for inhibition of maximal Kv3 whole cell conductance (G$_{max}$) by enflurane, isoflurane, halothane, and desflurane. The concentration of each volatile anesthetic agent was determined by gas chromatography and plotted against the inhibitory effect. IC$_{50}$ values and Hill coefficients are given in table 1.
This potency difference between the structural iso-
mers also is observed with Kv1.1 channels. En-
flurane inhibits Kv1.1 and Kv3 channels with nearly identical
IC50 values, whereas iso
flurane inhibits Kv1.1 channels
with an IC50 value of twice the concentration necessary
for half-maximal inhibition of Kv3 channels. Hill coef-
fi-cients differ between Kv3 channels and Kv1.1 channels
for inhibition by both anesthetics. As a consequence,
inhibition of Kv1.1 channels at clinical concentrations
would only become obvious with en-
flurane (2% at
2 MAC and 8% at 3 MAC).

The smaller than half-maximal effects of the volatile
anesthetics at clinical concentrations may suggest that
inhibition of voltage-dependent K channels does not
contribute to the pharmacologic action of these drugs
during clinical anesthesia. However, a concentration-
response curve at the cellular level needs not to be
identical with the concentration-response curve of an
intact neuronal network.11,12 A recent mutational study,
for example, shows that voltage-dependent K channels
determine the response of a specific brain circuit of
drosophila to halothane.13 Therefore, the relative lack of
sensitivity does not preclude voltage-dependent K chan-
nels from constituting a relevant biophysical target of
anesthetic agents.

In this context, it may be interesting that inhibition of
all 12 general anesthetics investigated on Kv3 channels
(this study and that of Friederich and Urban5) correlates
with clinical concentrations. This correlation is even
better than the correlation of K channel inhibition and
octanol–water coefficients14–16 (r = 0.97 vs. r = 0.75,
n = 12). Therefore, lipophilicity alone seems not to
predict the molecular actions of these drugs on Kv chan-
nels. Consequently, additional factors, such as specific
polar interactions, must be involved in the observed
inhibitory effects.17

In summary, volatile anesthetics inhibit human Kv3
and Kv1.1 channels in a concentration-dependent and
reversible manner. Pharmacologic effects already occur
at clinical concentrations. Whether inhibition of this
class of ion channels by volatile anesthetics may contrib-
ute to clinically observed anesthetic drug effects needs
further study.

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Fig. 2. (A) Superimposed traces of human
e voltage-dependent Kv1.1 currents evoked
by depolarizing steps from a holding poten-
tial of −80 mV to test potentials ranging
from −70 mV to +50 mV. The interpulse
duration was 1 s. Shown are the current traces during control conditions, during the
influence of enflurane (3.124 µM) and iso
flurane (4.037 µM), and after the drug
was washed out. (B) Concentration re-
sponse curves for inhibition of maximal
Kv1.1 whole cell conductance (Gmax) by en-
flurane and iso
flurane. The concentration
of each volatile anesthetic agent was deter-
mined by gas chromatography and plotted
against the inhibitory effect. IC50 values and
Hill coefficients are given in the Results.

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References


